10th Annual Biophysics Symposium
Pancoe-NSUHS Abbott Auditorium
Friday, September 13th, 2019 | 8 AM – 5 PM

Lila Giersch
University of Massachusetts Amherst
Mechanism of Hsp70 Molecular Chaperones: Allostery and Functional Tunability

Daniel Herschlag
Stanford University
How RNA Folds, and Why You Should Care

Benjamin A. Garcia
University of Pennsylvania School of Medicine
Quantitative Proteomics for Understanding Cancer Epigenetics

Susan Marqusee
University of California, Berkeley
Protein Folding On and Off the Ribosome

Julie Theriot
University of Washington
The Fast and the Furious: Mechanics and Dynamics of Rapid Cell Motility

Sponsored by
The Molecular Biophysics Training Program and The Structural Biology & Biophysics Cluster
10th Annual Biophysics Symposium
Organized by the Molecular Biophysics Training Program at Northwestern University

Program Schedule (page 1)

Friday, September 13th

7:45-8:00  Registration check-in (Pancoe Lobby, Pancoe 1st floor)
8:00-8:30  Breakfast (Pancoe Lobby, Pancoe 1st floor)

Session 1 (Pancoe Auditorium, Pancoe 1st floor)
Session Chair: Lauren Adams, MBTP Trainee (Kelleher Lab) and Co-Chair of Symposium Organizing Committee

8:30-8:35  Welcome Address by Michael Schamber, MBTP Trainee (Vafabakhsh lab) and Co-Chair of Symposium Organizing Committee
8:35-9:15  Distinguished Speaker: Lila Gierasch, PhD, University of Massachusetts, Amherst
          Mechanism of Hsp70 Molecular Chaperones: Allostery and Functional Tunability
9:15-9:30  Ryan Abdella, Past MBTP Trainee and IBiS Student (He lab)
9:30-9:45  Maggie Boyd, MBTP Trainee, Biomedical Engineering Student (Kamat lab)
9:45-10:00  Coffee break (Pancoe Lobby, Pancoe 1st floor)

Session 2 (Pancoe Auditorium, Pancoe 1st floor)
Session Chair: Julie Ming Liang, MBTP Trainee (Tullman-Ercek lab) and Co-Chair of Symposium Organizing Committee

10:00-10:05  Presentation of Dr. Susan Marqusee, Distinguished speaker by Julie Ming Liang
10:05-10:45  Distinguished Speaker: Susan Marqusee, PhD, University of California, Berkeley
             Protein Folding On and Off the Ribosome
10:45-10:50  Presentation of Dr. Benjamin Garcia, Distinguished speaker by Alexis Reyes
10:50-11:30  Distinguished Speaker: Benjamin Garcia, PhD, University of Pennsylvania
             Quantitative Proteomics for Understanding Cancer Epigenetics
11:30-11:45  Michael Schamber, MBTP Trainee and IBiS Student (Vafabakhsh lab)
11:45-12:00  Katherine Berman, IBiS Student (Lucks lab)
12:00-1:15  Lunch (Pancoe Café, Pancoe 2nd floor)

Session 3 (Pancoe Auditorium, Pancoe 1st floor)
Session Chair: Miranda Jacobs, MBTP Trainee (Kamat lab)

1:15-1:20  Presentation of Dr. Julie Theriot, Distinguished speaker by Miranda Jacobs
1:20-2:00  Distinguished Speaker: Julie Theriot, PhD, University of Washington
          The Fast and the Furious: Mechanics and Dynamics of Rapid Cell Motility
2:00-2:15  Thomas Wytock, PhD, Postdoctoral Fellow (Motter lab)
2:15-2:30  Denis Leshchev, PhD, Postdoctoral Fellow (Chen lab)
10th Annual Biophysics Symposium
Organized by the Molecular Biophysics Training Program at Northwestern University

Program Schedule (page 2)

Session 4 (Pancoe Café/ Pancoe Auditorium)
Session Chair: Luyi Cheng, MBTP Trainee (Lucks lab)

2:30-4:00    Poster session/Coffee break: Presentations by students and post-docs (Pancoe café, Pancoe 2nd floor)
4:00-4:05    Presentation of Dr. Daniel Herschlag, Distinguished speaker by Luyi Cheng
4:05-4:45    Distinguished Speaker: Daniel Herschlag, PhD, Stanford University
              How RNA Folds, and Why You Should Care
4:45-5:00    Awards presentation by Michael Schamber & Closing Remarks by Ishwar Radhakrishnan, MBTP Director
5:00 pm      Depart from Evanston
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On behalf of the NIH-supported Molecular Biophysics Training Program and the Structural Biology & Biophysics Cluster, I am pleased to extend a warm welcome to the 10th edition of the Annual Biophysics Symposium. We have reached a special milestone for this event – 10 years of bringing some of the most distinguished leaders in the field to share their passion for science while also providing a platform for our students and post-docs to share their exciting research! This day-long event, which brings the entire Northwestern biophysics community under one roof, was initiated at the behest of our student trainees, who each year take the lead role in organizing the event. This year Lauren Adams (Kelleher lab), Julie Liang (Tullman-Ercek lab), and Michael Schamber (Vafabakhsh lab) agreed to carry the torch as Co-Chairs of the Organizing Committee with Miranda Jacobs (Kamat lab), Alexis Reyes (He lab), and Luyi Cheng (Lucks lab), lending additional support, and Selma Aburahmeh, Program Coordinator and Assistant Research Administrator in the Department of Molecular Biosciences, providing key logistical support for the event.

The day-long event showcases ongoing biophysics research in 31 program preceptor laboratories spread over seven departments, six PhD programs, and three schools on the Evanston and Chicago campuses. The program schedule features podium and poster presentations post-doctoral fellows and graduate students including current and past trainees of the training program. We are once again very fortunate and deeply honored to have five distinguished scientists and mentors this year including Professors Benjamin Garcia, Lila Gierasch, Daniel Herschlag, Susan Marqusee, and Julie Theriot gracing this event. We are grateful to them for kindly agreeing to participate and generously sharing their science, knowledge, and wisdom with our students, post-docs, and faculty.

The event would not be possible without the financial backing provided by The Graduate School and the Molecular Biophysics Training Grant sponsored by the NIGMS.

I hope you will enjoy this event and through your participation contribute to its success. I also hope that you will continue to make the event an integral part of your annual calendar, as for the first time we are holding this event at the beginning of the school year (and will continue to do so moving forward).

Thank you for your support!

Ishwar Radhakrishnan
Director, Molecular Biophysics Training Program
Co-Director, Structural Biology & Biophysics Cluster
Benjamin A. Garcia, Ph.D.
John McCrea Dickson M.D. Presidential Professor,
Department of Biochemistry and Biophysics, Epigenetics Institute
University of Pennsylvania Perelman School of Medicine

Professor Benjamin Garcia received his B.S. in Chemistry from the University of California, Davis. He went on to receive his Ph.D. in Chemistry at the University of Virginia Charlottesville in 2005 under the mentorship of Dr. Donald F. Hunt, where his thesis work focused on quantitative mass spectrometry-based proteomics. He then pursued an NIH post-doctoral fellowship at the University of Illinois, Urbana-Champaign, with Dr. Neil Kelleher from 2005-2008.

Professor Garcia set up his own independent research program as an Assistant Professor in the Department of Molecular Biology at Princeton University in 2008, until his recruitment as the Presidential Associate Professor of Biochemistry and Biophysics at the University of Pennsylvania Perelman School of Medicine in 2012. He was then named the McCrea Dickson M.D. Presidential Professor in 2018. His research interests include using quantitative proteomics to characterize highly modified proteins, especially those involved in epigenetic mechanisms. His laboratory develops Bottom Up, Middle Down and time-resolved methods for rapid quantification of post-translational modifications (PTMs) in histones, histone variants and transcription factors.

Professor Garcia has been recognized with numerous awards and honors including an NIH Director's New Innovator Award, the Presidential Early Career Award for Scientists and Engineers (PECASE), the Ken Standing Award, the American Chemical Society Findeis Award, and the prestigious American Society for Mass Spectrometry Biemann Medal in 2018.
Lila M. Gierasch, Ph.D.
Distinguished Professor of Biochemistry and Molecular Biology and Chemistry
University of Massachusetts Amherst

Professor Lila Gierasch received her A.B. in Chemistry from Mount Holyoke College. She went on to receive her Ph.D. in Biophysics at Harvard University under the mentorship of Professor E.R. Blout.

Professor Gierasch set up her own independent research program as an Assistant Professor of Chemistry at Amherst College in 1974. She moved in 1979 to the University of Delaware and became a full professor there. In 1988, she was recruited as the Robert A. Welch Chair in Biochemistry at the University of Texas Southwestern Medical Center. She then joined University of Massachusetts, Amherst as the head of the Department of Chemistry in 1994 and then became the head of the Department of Biochemistry and Molecular Biology in 1999, before her appointment as a distinguished professor in 2006. Her research interests include studying the conformational preferences of model peptides to explore how local sequence guides folding, the in vitro folding of a predominantly β-sheet protein with a very simple topology, and how a protein folds in vivo. The methodology used in her lab include circular dichroism, fluorescence, and nuclear magnetic resonance spectroscopies, among other approaches.

For her outstanding research throughout her career, Professor Gierasch has been recognized with numerous accolades including the American Peptide Society Merrifield Award, the ASBMB Mildred Cohn Award, the Dorothy Crowfoot Hodgkin Award, and the American Chemical Society Ralph F. Hirschmann Award in Peptide Chemistry, among others. She is an elected member of the American Academy of Arts & Sciences and also the National Academy of Science, and is the current Editor-in-Chief for the Journal of Biological Chemistry.
Daniel Herschlag, Ph.D.
Professor of Biochemistry and Chemical Engineering
Stanford University School of Medicine

Professor Dan Herschlag received his B.S. in Biochemistry from SUNY-Binghamton and went on to receive his Ph.D. in Biochemistry at Brandeis University under the direction of Dr. W.P. Jencks, where his thesis work focused on the mechanisms of phosphoryl transfers. He is now a Professor of Biochemistry at the Stanford University School of Medicine, where he is also a faculty member in the Departments of Chemistry and Chemical Engineering.

Professor Herschlag's laboratory seeks to develop an understanding of the fundamental behavior and roles of RNA and proteins in biology. His work focuses on RNA structure, folding and catalysis, as well as RNA/protein interactions in regulation and control. His lab uses a wide range of techniques spanning single molecule fluorescence, small angle X-ray scattering, atomic emission spectroscopy, and solution NMR spectroscopy.

During his illustrious career, Professor Herschlag has been recognized with numerous awards including multiple mentoring and service awards from Stanford University, the NIH Merit Award, the Pfizer Award in Enzyme Chemistry and Cope Scholar Award from the American Chemical Society, among others. He is an elected member of the National Academy of Sciences and has also served as the Senior Associate Dean of Graduate Education and Postdoctoral Affairs at Stanford University School of Medicine.
Susan Marqusee, M.D., Ph.D.
Eveland Warren Endowed Chair
Professor of Molecular and Cell Biology & Chemistry
University of California, Berkeley

Professor Susan Marqusee received her A.B. in Chemistry and Physics at Cornell University and went on to earn her M.D./Ph.D at the Stanford University School of Medicine and the Department of Biochemistry. Her thesis work in Dr. Robert L. Baldwin’s lab directly tackled the protein folding problem and led to the first ever de novo design of a short peptide that folded into a specific structure, an alpha helix. Her work sought to understand the energy dynamics behind the formation of alpha helices from these alanine-based peptides which were not predicted to form alpha helical secondary structures. She is now a Professor in the Molecular & Cell Biology and Chemistry departments at University of California, Berkeley. In addition, she is the Berkeley director of the California Institute for Quantitative Biosciences (QB3), a non-profit collaborative research institute of over 200 faculty researchers in the San Francisco Bay Area.

Professor Marqusee is a top protein folding experimentalist and her laboratory continues on the theme of her thesis research by seeking to understand the 3D structures that form from a linear 1D amino acid sequence. Studying how a protein sequence determines the energy landscape of a protein is necessary in understanding how the protein folds and the biological processes the protein carries out. Her laboratory uses a combination of biophysical, structural, and computational methods to accomplish their research. Some techniques include hydrogen exchange methods to study rare partially structured conformers, use of optical tweezers in single molecule force spectroscopy (SMFS) to detect protein folding pathways and intermediates, and computational modelling of translation and folding.

Professor Marqusee has won numerous awards throughout her career including the Beckman Young Investigator award, the Margaret Dayhoff Oakley award from the Biophysical Society, the William Rose Award from the American Society of Biochemistry and Molecular Biology, the Dorothy Crowfoot Hodgkin Award from Protein Society, and the Arthur Kornberg and Paul Berg Lifetime Achievement Award in Biomedical Sciences from the Stanford Medical School Alumni Association. For her many contributions to science, she has been elected as a Fellow of the American Academy of Arts and Sciences and a Member of the National Academy of Sciences.
Julie Theriot, Ph.D.
Benjamin D. Hall Endowed Chair
Professor of Biology
University of Washington

Professor Julie Theriot earned a B.S. in Physics and a B.S. in Biology from the Massachusetts Institute of Technology in 1988. She then went on to receive her Ph.D. in Cell Biology in 1993 from University of California, San Francisco where she began her research on actin motility in diverse cell types from pathogenic bacteria to fibroblasts and fish epidermal keratocytes. She continued this work on cellular motility at the Whitehead institute as a Whitehead Fellow.

In 1997, Professor Theriot became an Assistant Professor at Stanford in the Departments of Biochemistry and of Microbiology & Immunology. In 2018, she moved her research lab to the Department of Biology at the University of Washington and is currently a Howard Hughes Medical Institute Investigator. Her research is highly interdisciplinary and focuses broadly on understanding cellular self-organization and motility in many diverse cell types. Specifically, she is working to understand the motility of intracellular bacterial pathogens through actin, and the whole-cell crawling of epithelial cells and leukocytes. Additionally, her group is studying the spatial and temporal dynamics of cellular self-organization in bacteria and diatoms.

Professor Theriot has been recognized for her scientific achievements through numerous awards throughout her career. Some of her honors include the John D. and Catherine T. MacArthur Foundation Fellowship and the David and Lucile Packard Foundation Fellowship for Science and Engineering. In 2016, Professor Theriot was appointed as an American Society for Cell Biology Lifetime Achievement Fellow and was recruited as the Benjamin D. Hall Endowed Chair in Basic Life Sciences at the University of Washington in 2018.
Histones are small proteins that package DNA into chromosomes, and a large number of studies have showed that several post-translational modification (PTM) sites on the histones are associated with both gene activation and silencing. Along with DNA and small non-coding RNA, histone PTMs make up epigenetic mechanisms that control gene expression patterns outside of DNA sequence mutations. Dysregulation of these chromatin networks underlie several human diseases such as cancer. Here I will give an update on technology advancements that have allowed for high-throughput quantitative analyses of histone PTMs and chromatin structure, and how we are applying these methods to understand epigenetic reprogramming found in malignant peripheral nerve sheath tumors (MPNSTs). MPNST is an aggressive sarcoma with recurrent loss of function alterations in polycomb-repressive complex 2 (PRC2), a histone-modifying complex involved in transcriptional silencing.
Mechanism of Hsp70 Molecular Chaperones: 
Allostery and Functional Tunability  

Lila M. Giersch, Ph.D.

The Hsp70 family of chaperones works with its co-chaperones, the nucleotide exchange factors (NEFs) and J-proteins, to facilitate a multitude of cellular functions. Central players in protein homeostasis, these jacks-of-many-trades are utilized in a variety of ways because of their ability to bind with selective promiscuity to regions of their client proteins that are only exposed when the client is unfolded, either fully or partially, or visits a conformational state that exposes the binding region in a regulated manner. The key to Hsp70 functions is that their substrate binding is transient and allosterically cycles in a nucleotide-dependent fashion between high and low affinity states. In the past few years, structural insights into the molecular mechanism of this allosterically regulated binding have emerged and provided deep insight into the deceptively simple Hsp70 molecular machine. The ability of each of the two Hsp70 structural domains—the N-terminal nucleotide-binding domain and the C-terminal substrate-binding domain—to switch between two conformational states in a ligand-gated manner, and the built-in mechanism of interdomain communication that triggers these conformational switches, are key to Hsp70 action. The allosteric energy landscape of Hsp70s can be sculpted by amino acid substitutions, which leads to a tunability that has been evolutionarily exploited. Because of their importance in maintaining cellular homeostasis, there is great interest in modulating Hsp70s with small molecules to address diseases arising from loss of homeostasis. Structural insights into their molecular mechanism provide essential understanding for any rational design of modulators.
How RNA folds, and why you should care

Daniel Herschlag, Ph.D.

The discovery of catalytic RNA fueled interest in RNA as a functional, structured molecule in modern biology and as a possible solution to the “chicken-and-egg” problem of how genetic transmission and catalytic function could co-evolve. In the intervening decades it has become clear that structured RNAs function throughout biology and that RNA structure–function is relevant to many diseases. There is also a growing interest in RNA therapeutic targets.

Science progresses from discovery, through characterization, ultimately to predictive models. Recent conceptual breakthroughs have led to the RNA Reconstitution Hypothesis and recent experimental breakthroughs have provided strong support for this hypothesis and methodologies to determine the molecular behavior of 1000s of RNA elements in parallel, as is needed to develop a predictive understanding of RNA dynamics and thermodynamics.

Most broadly, we seek to describe biology in its natural language, that of the chemical and physical properties of its molecular constituents. These properties define the capabilities and limitations of biological systems, and represent an increasingly important perspective required for deep understanding and evaluating and making sense of the immense influx of data in the genomic era.

Schematic of progress in a scientific field in terms of “epochs”
Protein Folding On and Off the Ribosome

Susan Marqusee, M.D., Ph.D.

Understanding the structural and dynamic information encoded in the primary sequence of a protein is one of the most fundamental challenges in modern biology. The amino acid sequence of a protein encodes more than the native three-dimensional structure; it encodes the entire energy landscape – an ensemble of conformations whose energetics and dynamics are finely tuned for folding, binding and activity. Small variations in the sequence and environment modulate this landscape and can have effects that range from undetectable to pathological. I will present our recent results probing these sequence and environmental effects using a combination of single-molecule and ensemble-based studies.
The Fast and the Furious: Mechanics and Dynamics of Rapid Cell Motility

Julie Theriot, Ph.D.

Directed crawling motility of animal cell types ranging from neurons to macrophages requires the coordinated force-generating activity of multiple mechanical elements. Much molecular detail is now known about the constituents of some mechanical submachines such as the polymerizing actin network and the adhesion complexes, but it is not yet clear how these elements all work together to generate coherent, directed motion at the level of the whole cell. In order to understand cellular mechanisms of large-scale coordination, our work focuses on two extremely fast-moving cell types, the fish epidermal basal keratocyte responsible for the rapid closure of wounds in fish skin, and the human neutrophil that hunts down and kills microbial invaders. Despite their very different biological roles and apparent behaviors, these cells share fundamental mechanisms of self-organization and movement coordination.
Structure of the parainfluenza virus 5 RNA polymerase complex by cryo-electron microscopy

Ryan Abdella¹, Megha Agarwhal², Takashi Okura², Robert Lamb¹,², and Yuan He¹

¹Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208
²Howard Hughes Medical Institute, Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208

The large (L) protein of paramyxoviruses is responsible for duplicating the virus’ RNA genome and synthesizing viral mRNA. To accomplish this, the viral protein has adopted a modular structure composed of an RNA-dependent RNA polymerase (RdRp) for genome duplication and mRNA production, and capping (Cap) and methyl-transferase (MTD) domains for mRNA processing. In order to function in vivo, L requires a cofactor, the phosphoprotein (P), which facilitates access to the RNA contained within the viral nucleoprotein complex. Structural information is available for individual L and P proteins from different paramyxoviruses, but how P interacts with L and how that affects L activity is largely unknown due to the lack of high-resolution structures of these complexes. Here we report the structure of the L-P complex from parainfluenza virus 5 (PIV5) by single particle cryo-electron microscopy (EM), as well as an x-ray crystallographic structure of the oligomerization domain (OD) of P. P forms a tetramer with the ODs forming a parallel four helix bundle that interacts with L at the base of the RdRp domain. A single X domain (XD) domain from the P tetramer, sufficient for P to interact with L, is bound near the nucleotide entry site of the RdRp domain. Compared to the only known structure of an L protein from vesicular stomatitis virus (VSV), the MTD and C-terminal domains (CTD), which together form the active site for methylation, are on the opposite side of the connecting domain (CD), positioning the MTD active site immediately above the capping domain (Cap) and near the likely exit site for the product RNA 5’ end. We have also identified a subtle rotation of the CD-MTD-CTD module away from the rest of the complex; flexibility in this region is necessary to allow a conformation change between the VSV and PIV5 structures. We propose that the PIV5 conformation represents an mRNA synthesizing-competent complex and the VSV structure is a genome duplication-competent complex.
Optical dyes to monitor tension and growth in model membranes

Margrethe Boyd, Neha Kamat

Department of Biomedical Engineering, Northwestern University, Evanston, IL 60208

Cells have been observed to respond to various physical cues by dynamically regulating the mechanical properties of the cell membrane. In particular, the force of membrane tension has been shown to cause changes in membrane surface area, a process which plays a role in cellular growth, migration and signaling. The ability to monitor changes in tension and surface area simultaneously has the potential to offer new insights into the biophysical processes underlying this relationship, yet we lack robust methods to accomplish this. Here, we introduce a set of spatially distinct optical probes that allow real time observations of membrane strain and growth. Localized to the hydrophilic head region, phospholipid-conjugated FRET pairs report changes in surface area as membranes expand. Laurdan, a polarity-sensitive probe localized to the hydrophobic interior, responds as water molecules penetrate the stretched bilayer. These dyes act in concert to monitor changes in lipid separation and membrane polarity, exhibiting shifts in fluorescent output as membrane properties vary over time. Using this system in model membranes, we observed a linear relationship between tension and fluorescent output for both types of dyes. Additionally, these probes allowed us to monitor the process of tension-induced lipid uptake, showing that membranes under strain take up excess lipid faster and in greater amounts than their non-tensed counterparts. Ultimately, through this combinatorial use of spatially distinct optical probes, we can simultaneously observe dynamic changes that occur in membranes under tension, allowing us to obtain a better picture of the role and nature of these changes.
Mechanism of activation and modulation of the Calcium-Sensing Receptor

Michael Schamber, Reza Vafabakhsh

Molecular Biosciences, Interdisciplinary Biological Sciences, Northwestern University, Evanston, IL 60208

The calcium-sensing receptor (CasR) is a class C G-protein coupled receptor (GPCR) with a large ectodomain (ECD) comprised of the venus flytrap ligand binding domain (LBD) and a structured cysteine-rich domain (CRD) linking the LBD to the prototypical seven transmembrane (7TM) domain. In the canonical model of class C activation, ligand binding induces a local conformational change and global structural rearrangement of the ECD which is relayed over 12 nm to allosterically activate the G protein-binding interface. Surprisingly, CasR signaling has been shown to depend on both calcium and amino acids. However, whether each ligand can individually activate the receptor has been controversial. Moreover, CasR has been shown to be a pleotropic receptor capable of activating different G protein pathways. Currently a structural understanding of this phenomena is lacking. Despite CasR’s ability to bind amino acids in the cleft, they are unable to activate the receptor without Ca$^{2+}$. Because of this, it is uncertain whether CasR follows the canonical model as previously suggested or if it has a novel mechanism. Recent crystal structures of truncated CasR fail to capture CasR in the presence of calcium alone leaving this question unanswered. Furthermore, static structures are unable show the dynamic process of conformational change – failing to capture critical intermediates. What is missing is the contribution of amino acids and calcium to the activation process of CasR. Mechanistic model for the activation of CasR.

The most direct way to answer these questions is to watch full-length CasR as it undergoes activation in real-time. We have developed a single-molecule FRET (smFRET) assay to do this, which enabled us to map the conformational landscape of CasR in the presence of different ligands. We find minimal conformational change of the upper lobe of CasR’s LBD upon activation in stark contrast to the metabotropic glutamate receptors (mGluRs), another member of class C GPCRs. We also identify novel FRET states corresponding to intermediate conformations of the CRD in addition to states that correspond to already identified inactive and active conformations. Surprisingly, we find that L-Trp stabilizes CasR’s LBD more potently than Ca$^{2+}$ alone. However, for the CRD, L-Trp stabilizes an intermediate conformation that corresponds to flytrap closure. Taken together our results suggest amino acids act as allosteric modulators by facilitating closure of the LBD, which is a necessary step for CasR activation.
Cotranscriptional RNA strand invasion mediates ligand sensing in the *E. coli thiB* thiamine pyrophosphate riboswitch

**Katherine Berman**¹ and Julius Lucks²

¹Interdisciplinary Biological Sciences Graduate Program, Northwestern University, Evanston, IL 60208
²Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL 60208

RNA folds immediately as it is transcribed by RNA polymerase, thereby creating RNA structures faster than new nucleotides being added to an emerging RNA chain. Consequently, cotranscriptionally folded RNA structures can differ greatly from equilibrium folded structures. Riboswitches are structured RNA elements that respond to a variety of small molecules, ions, and other metabolites to regulate transcription, translation, splicing, and mRNA degradation. Riboswitches take advantage of cotranscriptional RNA folding pathways to regulate gene expression by altering the folding pathway depending on the presence or absence of a ligand. The thiamine pyrophosphate (TPP) riboswitch, a highly conserved riboswitch family found in all kingdoms of life, has been shown to regulate translation via changes in cotranscriptional RNA structure. In this work, we have determined the *Escherichia coli thiB* TPP riboswitch uses strand invasion to sense TPP binding and form a ‘sequestering stem’ around the ribosome binding site. To examine this mechanism, we have used cotranscriptional SHAPE-seq, a technique which combines RNA chemical probing, precise polymerase arrest and high-throughput sequencing, to study intermediate structures in the folding pathway of the *E. coli thiB* TPP riboswitch. This data revealed an intermediate hairpin which senses ligand binding by strand displacement coordinated by the ligand binding site. A series of deletion mutants created to favor or disfavor this intermediate structure demonstrated efficient strand invasion to be essential to *thiB* riboswitch function. This work along with recent work on the ZTP riboswitch and the signal recognition particle (SRP) RNA have demonstrated the prevalence of strand invasion in RNA cotranscriptional folding pathways.
Revealing Protein Structural Dynamics Using X-Ray Scattering and Environmental Perturbations

Denis Leshchev, Darren Hsu, Arnold Chan, Lin Chen

Chemistry Department, Northwestern University, Evanston, IL 60208

Over the past two decades, pump-probe time-resolved x-ray solution scattering (TRXSS) has emerged as a powerful method capable of direct tracking of tertiary and secondary structural changes in proteins on the time scales ranging from femtoseconds to seconds. Up until recently, TRXSS applicability was limited only to light-sensitive proteins, i.e. to systems where dynamics can be initiated by direct excitation of protein’s intrinsic chromophore with light. We have recently demonstrated how an indirect excitation of proteins, such as laser-induced temperature and pH jumps, can be coupled to TRXSS to study dynamics of proteins that are not light sensitive. Here we show the latest TRXSS results on elucidating insulin dimer dissociation, as well as observations on hierarchical structural transformations in poly-L-glutamic acid. In case of both systems, TRXSS provides new insights into macromolecular conformational dynamics by indicating presence of structural intermediates that could not be observed with other methods. The new methods of indirect protein excitation greatly expand the applicability of TRXSS towards non-photoactive biosystems. This allows for future investigations of a broad range of biological processes with high structural sensitivity and high temporal resolution at the same time.
Predicting Growth Rate from Gene Expression

Thomas P. Wytock, Adilson Motter

Department of Physics and Astronomy, Northwestern University, Evanston, IL 60208

Growth rate is one of the most important and most complex phenotypic characteristics of unicellular microorganisms, which determines the genetic mutations that dominate at the population level, and ultimately whether the population will survive. Translating changes at the genetic level to their growth rate consequences remains a subject of intense interest, since such a mapping could rationally direct experiments to optimize antibiotic efficacy or bioreactor productivity. In this paper, we directly map transcriptional profiles to growth rates by gathering published gene-expression data from *Escherichia coli* and *Saccharomyces cerevisiae* with corresponding growth-rate measurements. Using a machine-learning technique called $k$-nearest-neighbors regression, we build a model which predicts growth rate from gene expression. By exploiting the correlated nature of gene expression and sparsifying the model, we capture 81% of the variance in growth rate of the *E. coli* dataset while reducing the number of features from over 4,000 to nine. In *S. cerevisiae*, we account for 89% of the variance in growth rate while reducing from over 5,500 dimensions to 18. Such a model provides a basis for selecting successful strategies from among the combinatorial number of experimental possibilities when attempting to optimize complex phenotypic traits like growth rate.
Molecular Mechanism of Ligand-Independent Activation by Nuclear Receptors

Nicolas Daffern, Yongbo Zhang, Ishwar Radhakrishnan

Molecular Biosciences, IBiS, Northwestern University, Evanston, IL 60208

Nuclear receptors (NRs) are an important family of transcription factors in metazoans that control a broad range of fundamental physiological processes in response to diverse environmental cues. Since most receptors bind and respond to small molecule ligands and regulate specific cellular pathways, they also provide opportunities for therapeutic intervention. Not surprisingly, almost one out of six therapeutics in the clinic act on one of 48 NRs as their primary target. NRs harbor two conserved domains, a ligand-binding domain and a DNA-binding domain, whose roles in NR function have been generally well-characterized. However, most NRs also feature a relatively less-conserved transactivation domain called AF1 that is poorly characterized. This domain has been implicated in ligand-independent activation mechanisms, but whose molecular and structural basis remain obscure.

To address this gap in knowledge, we used bioinformatics in conjunction with biochemical and biophysical approaches to identify a novel interactor for the AF1 domain of multiple NRs and characterize its interaction with the AF1 domains. Specifically, our GST-pulldown and NMR studies identified the PAS-B domain of the steroid receptor coactivator, SRC1, as a candidate for recruitment by the AF1 domains of Nurrl, a member of the NR4A subfamily, and the androgen receptor, a member of the steroid receptor subfamily. I will present ongoing work in the lab that is focused on structural and functional studies of these interactions using solution NMR spectroscopy and other biophysical methods. These studies are the first known instances of structural characterization of AF1 domains and represent an important step forward in delineating the mechanism by which these transactivation domains exert their role in NR function.
A mechanistic study of the *pbuE* adenine riboswitch from the perspective of cotranscriptional chemical probing

**Luyi Cheng**\(^1\), Robert T. Batey\(^2\) and Julius B. Lucks\(^3\)

\(^1\)Interdisciplinary Biological Sciences Graduate Program, Northwestern University, Evanston, IL 60208  
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Riboswitches are non-coding RNAs that create unique RNA-based regulatory systems found in all domains of life and especially prevalent in bacteria. Riboswitch sequences regulate genes in response to specific environmental factors such as small molecule toxins, chemical alarmones, and metabolic cofactors. To regulate these genes, riboswitches respond to ligand binding by folding into structures that control transcription, translation, or RNA cleavage. Among the 40 diverse classes of known riboswitches, a subset regulates transcription by making their regulatory decisions during the fast timescales of transcription, requiring structures to fold cotranscriptionally. While we have a mature understanding of the chemical basis of RNA-ligand interactions and the structural characteristics of riboswitch aptamers, we lack a general understanding of the step-by-step structural transitions that occur during cotranscriptional folding that enable rapid genetic decisions. Here, I use the *Bacillus subtilis pbuE* adenine riboswitch as a model system to examine how cotranscriptional folding events and intermediate structures lead to functional gene expression outcomes. To understand this process, I optimize and apply cotranscriptional SHAPE-Seq, a technique that combines chemical probing and next generation sequencing to uncover the ligand-dependent folding pathway of the *pbuE* adenine riboswitch at a single nucleotide resolution. We specifically examine the structural order of events for aptamer folding, terminator nucleation and strand invasion by the expression platform. We aim to use these results, in combination with recent studies on the ZTP and fluoride riboswitches, to further develop hypotheses for more generalized principles of the role of cotranscriptional structures in riboswitch gene regulation.
Nuclear Pore Interaction Confines Chromatin Movement

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Throughout a cell’s life cycle, its genome undergoes significant physical restructuring and molecular modification. These genomic changes affect genetic regulation both directly and indirectly. For example, activating a repressed gene in a eukaryotic organism requires a series of coordinated changes in protein occupancy, nucleosome modifications, and local chromatin structure. In \textit{S. cerevisiae}, commonly known as budding or brewer’s yeast, transcriptional activation commonly includes the formerly repressed genetic locus translocating from the nucleoplasmic space to the nuclear periphery. This change in positioning is regulated through a variety of mechanisms specific to the stimulus inducing activation and the targeted gene. All of these regulatory mechanisms require at least one of a series of transcription factors referred to as “positioning factors.” A majority of transcription factors in \textit{S. cerevisiae} can act as positioning factors and have been shown to be sufficient for peripheral localization when artificially tethered at an ectopic locus. While there are many putative positioning factors, all positioning factor mediated gene relocalization requires the same nuclear pore proteins to establish and maintain positioning at the nuclear periphery. The diffusion rate of a gene localized at the nuclear periphery is significantly more confined both in average step length and the dynamic range of movement when compared to a gene in the nucleoplasmic space. Despite this constraint on diffusion, genes at the periphery maintain a displacement rate higher than membrane embedded nuclear structures, such as the nuclear pore complex, and undergo interchromosomal clustering with other active genes. Utilizing high spatio-temporal resolution microscopy, displacement analysis, and an optogenetic positioning factor, we are able to gain insight into the interactions and mechanisms that control chromatin translocation, gene positioning, and interchromosomal clustering.
Micropipette isolation of mitotic chromosomes and whole-genome bundles to study the effects of chromatin-organizing proteins depletion

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Mitotic chromosomes are dense thread-like structures of compact chromatin. These structures are entangled with each other in mitosis and can be further isolated with micropipettes and physically manipulated to calculate their physical properties. Here we present three projects using this technique to study protein stability on mitotic chromosomes and the stiffness of chromosomes and centromeres. In the first project, we probe the effects of histone PTMs in the underlying chromatin on the stiffness of the whole mitotic chromosomes using PTM modifying drugs. We show that hyperacetylation of histones does not affect the stiffness of mitotic chromosomes, but hypermethylation causes an 80% increase in stiffness. Second, we assess the stability of multiple complexes on mitotic chromosomes outside of the cell by photobleaching experiments on the mitotic genome bundle (a bundle of entangled mitotic chromosomes). We show that in our experiments, cohesin (Rad-21) fluorescence is immediately lost from the bundle, Ki-67 fluorescence is quickly lost from the bundle, and condensin (SMC-2) fluorescence is very stable over 2 hours of holding the bundle. Our work will continue in investigating the role of these complexes and more on the qualitative effect of the bundle, their fluorescent patterning on mitotic chromosomes the mechanical differences of their degradations in mitotic bundles and single chromosomes. Finally, we show that the centromere region of the centromere is substantially stiffer than the arms of mitotic chromosomes. Further experiments with this analysis will involve studying the effects of CENP-C, CENP-N, condensin, and cohesin depletion on the stiffness of the centromere.
X-ray Snapshots Reveal Conformational Influence on Active Site Ligation During Metalloprotein Folding

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Cytochrome c (cyt c) has long been utilized as a model system to study metalloprotein folding dynamics and the interplay between active site ligation and tertiary structure. However, recent reports regarding the weakness of the native Fe(II)-S bond (Fe-Met80) call into question the role of the active site ligation in the protein folding process. In order to investigate the interplay between protein conformation and active site structures, we directly tracked the evolution of both during a photolysis-induced folding reaction using X-ray transient absorption spectroscopy and time-resolved X-ray solution scattering techniques. We observe an intermediate Fe-Met80 species appearing on ~2 µs timescale, which should not be sustained without stabilization from the folded protein structure. We also observe the appearance of a new active site intermediate: a weakly interacting Fe-H₂O state. As both intermediates require stabilization of weak metal-ligand interactions, we surmise the existence of a local structure within the unfolded protein that protects and limits the movement of the ligands, similar to the entatic state found in the native cyt c fold. Furthermore, we observe that in some of the unfolded ensemble, the local stabilizing structure is lost, leading to expansion of the unfolded protein structure and misligation to His26/His33 residues.
Cryo-electron microscopy structure of a nucleosome-bound SWI/SNF chromatin remodeling complex

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The multi-subunit chromatin remodeling complex SWI/SNF is highly conserved from yeast to humans and plays critical roles in various cellular processes including transcription and DNA damage repair. It uses the energy from ATP hydrolysis to remodel the chromatin structure by sliding and evicting the histone octamer, creating DNA regions that becomes accessible to other essential protein complexes. However, our mechanistic understanding of the chromatin remodeling activity is hindered by the lack of high-resolution structure of this critical complex. Here we report the first structure of SWI/SNF from the yeast S. cerevisiae bound to a nucleosome at near atomic resolution determined by cryo-electron microscopy (cryo-EM). Our structure shows a unique feature that the Arp module is sandwiched between the ATPase and the Body module of the complex, with the HSA domain from Snf2 connecting the three. The HSA domain also extends into the Body and anchors at the opposite side of the complex. The Body contains an assembly scaffold composed of conserved subunits Snf12 (BAF60), Snf5 (BAF47) and an asymmetric dimer of Swi3 (BAF155/170). The other conserved subunit Swi1 (ARID1) folds into an Armadillo (ARM) repeat domain that resides in the core of the SWI/SNF Body, acting as a molecular hub. In addition to the interaction between Snf2 and the nucleosome, we also observed interactions between the conserved Snf5 subunit and the histones near the acidic patch, which could serve as an anchor point during active DNA translocation. Our structure allows us to map and rationalize some cancer related mutations in the human SWI/SNF complex and propose a model of how SWI/SNF recognizes and remolds the +1 nucleosome to generate nucleosome-depleted region during gene activation.
Structural and functional studies on Sap transporter system in NTHi

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During bacterial infections, the host utilizes various strategies to keep bacteria from accessing essential nutrients (metals), a process termed nutritional immunity. To avert the host's defenses, pathogenic bacteria have evolved a myriad of mechanisms to acquire these essential nutrients from the host. Bacterial transport system like ATP-binding cassette (ABC) transporters are necessary for nutrient uptake and is a major factor in host-pathogen interactions. The Sap (sensitivity to antimicrobial peptides) transporter from nontypeable *Haemophilus influenzae* (NTHi) is one such example with the ability to scavenge heme from the host while circumventing the effects of host-derived antimicrobial peptides (AMPs). The core of Sap transport system has a four domain architecture comprised of two transmembrane domains (SapB and SapC) that form a translocation channel and two nucleotide binding domains (SapD and SapF). The fifth component, the periplasmic binding protein SapA, delivers substrates to the core complex. The binding and hydrolysis of ATP in the NBDs powers the transport of nutrients across the inner membrane.

Although Sap function is required for full NTHi virulence, the molecular mechanism and structural aspects of Sap transporter are not still understood. 1) How do the NBDs form a complex with the TMDs to select and transport each substrate? 2) How is Sap transporter system regulated?

To investigate the functional assembly units of NBDs for the Sap transporter complex, we purified each of the Sap transporter components and determined the SapF structure by x-ray crystallography to 2.3Å resolution. Via protein engineering, we obtained monomeric SapD protein with a bound iron-sulfur cluster at its c-terminal regulatory region. Our SPR data and structural studies suggest that homodimeric NBDs (SapD-SapD and SapF-SapF) are functional units of the Sap complex. Negative stain micrographs of a Sap transporter complex, (SapC-SapF)2 showed monodisperse particles and representative 2D class average images, providing the first views of the Sap transporter as a Type I ABC complex. Structural and functional studies provide direct evidence for the homodimeric NBDs are the functional units of Sap transporter, and insights into how the regulatory region of the NBD may control Sap transport system.
Characterizing assembly and function of the Type Three Secretion System following different modes of activation

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With the advent of recombinant DNA technology, proteins can now be made in large-scale as therapeutics to treat diseases, enzymes for industrial processes, and even biomaterials like spider silk. Unfortunately, industrial-scale protein production can be inefficient as extensive purification is required in order to extract and purify the protein-of-interest (POI) from the production organism. We address this issue by engineering a protein secretion system, the Salmonella Pathogenicity Island-1 type three secretion system (SPI-1 T3SS), of Salmonella enterica to secrete POIs directly into the supernatant of the cell’s culture media.

The SPI-1 T3SS is natively used by Salmonella to invade and infect host organisms and its regulation is subject to many environmental conditions that activate the system by several different transcriptional regulators. As such, activation of T3SS can be induced by synthetically controlling these regulators. We are studying how activation of the T3SS by various induction mechanisms influence (1) SPI-1 T3SS gene expression, (2) assembly of the T3SS needle apparatuses, and (3) functional output in the form of protein secretion titer. This will expand our understanding of the steps between T3SS activation and invasion and will enable us to better engineer the T3SS for heterologous protein secretion.
Investigating the DNA-binding of homo- and hetero-dimers of the zinc cluster transcription factors Pdr1 & Pdr3

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Multidrug resistance (MDR) is an increasingly serious concern in the treatment of opportunistic fungal infections that are commonly found in immunocompromised individuals. A majority of the gene networks responsible for MDR are regulated by zinc cluster proteins, a family of transcription factors unique to fungal organisms. Growing evidence suggests these transcription factors are fungal analogs of metazoan nuclear receptors that allow fungi to respond to the presence or absence of small molecules. The transcription factors Pdr1 and Pdr3 co-regulate the expression of a group of ABC exporters responsible for MDR in *S. cerevisiae*, and have homologs in the MDR network of the most common fungal pathogen, *C. glabrata*. Despite their importance in regulating drug resistance, it remains unclear how Pdr1/Pdr3 recognize a toxic environment, the differences in their function, and how they work together due to a lack of structural and biochemical data.

Pdr1 and Pdr3 share the same DNA-binding motif and are capable of forming homo- or hetero-dimers bound to DNA with unknown differences in function. I have been investigating the DNA-binding properties of these two transcription factors to identify differences in DNA-binding affinity and what factors promote formation of either homo- or hetero-dimers. The ability to form several types of dimers in a context dependent manner may create another layer of transcriptional regulation beyond the presence of the required DNA-binding motif. Additional work has focused on determining their structures via x-ray crystallography to study the dimerization interface and the mechanism of transcription activation. Understanding how Pdr1/Pdr3 function together will provide insight on the general mechanisms of how zinc cluster proteins regulate transcription, and how drug resistance in fungal organisms may be disrupted.
Determining the Transient Structures of a Fast-Folding Mini-Protein

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The 20-residue Trp-cage synthetic mini-protein is one of the smallest systems to exhibit a stable secondary structure and fast-folding dynamics less than 4 µs. These properties make it amicable to detailed theoretical investigations of protein folding, which have been performed over past decades. While various works based on molecular dynamics simulations predicted complex folding behavior for Trp-cage, these models have not been confirmed experimentally. Few spectroscopic works have inferred a single stable intermediate conformation on a pathway from folded to unfolded basins; however, no structural information is available for this intermediate folding step. To bridge the understanding of secondary structure dynamics of Trp-cage on nanosecond- to microsecond-timescales, we use time-resolved X-ray solution scattering (TRXSS) to probe the temperature-induced unfolding of Trp-cage. Laser-initiated environmental perturbations coupled with X-ray scattering allow for the observation of transient structural dynamics during the folding/unfolding process. Our results indicate the formation of an unfolded intermediate on the timescale of <100 ns, which undergoes complete unfolding within 650 ns. The ongoing structural analysis paves the way for direct benchmarking of theoretical models of protein folding produced with molecular dynamics simulations, which in turn will provide deeper insight into the process of secondary structure formation.
Uncovering the role of membrane elasticity in membrane protein folding

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Membrane protein expression and folding into vesicle membranes is important for developing functional model cellular systems, bioreactors, and artificial cells. Many membrane proteins have been successfully incorporated into artificial membranes, however, the effects of membrane properties on protein insertion and oligomerization are not well understood. The incorporation of non-natural amphiphiles, such as diblock copolymers, into artificial membranes allows for fine tuning of membrane physical properties, which has not been possible using natural biological molecules alone. Here, we use cell free protein expression to observe cotranslational folding in response to membrane properties using a model mechanosensitive channel protein, the mechanosensitive channel of large conductance (MscL) with a C-terminal green fluorescent protein (GFP) fusion. Folding studies were conducted by expressing MscLGFP in the presence of small unilamellar phospholipid vesicles composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). Protein folding efficiency and total production were measured by monitoring GFP fluorescence and quantitative western blots respectively. We first observed that increasing the total available amphiphile concentration in a cell-free reaction increased the efficiency and yield of membrane protein folding. We then blended an amphiphile that reduces membrane area expansion modulus ($K_a$), poly(ethylene oxide)-b-poly(butadiene), into phospholipid membranes and observed the folding and total production of MscL improved beyond folding observed in pure phospholipid membranes. Finally, we demonstrated this effect is reproducible with an alternate amphiphile that also decreased membrane $K_a$, measured by micropipette aspiration, and observed enhanced protein folding with respect to pure lipid membranes. Overall, these results indicate that membrane physical properties—such as membrane surface area and stiffness—play a role in unassisted folding and expression of MscL in a cell free reaction. Moreover, our results introduce how non-natural amphiphiles may be used to uncover the impact of physical properties of membranes on membrane protein behaviors.
Dynamics in allostery for the bacterial fimbrial adhesion protein FimH

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Most urinary tract infections are caused by E. coli expressing the fimbrial adhesion protein, FimH, which binds ligand on urothelial cells to limit bacterial clearance during urination. During urination, shear flow separates the two domains of FimH, and this perturbation at the allostERIC, interdomain site induces a conformational change from the inactive to the active structure. A truncated mutant of the ligand-binding lectin domain lacks the second domain involved in allostery and has a single arginine-to-proline mutation (R60P), which is located on the putative allosteric pathway. The R60P mutation is distant from both the allosteric and ligand-binding sites. From the literature, the truncated mutant has a crystal structure matching the inactive structure but shows differences in binding kinetics and thermodynamics compared to inactive, full-length FimH. Here, we study how the R60P mutation and truncation changes fast dynamics at the nano-to-picosecond timescale using molecular dynamics (MD) simulations. To analyze time-series data from the simulations, we use tools from information theory and network reconstruction. We capture differences in the dynamics of the FimH lectin domain in the presence and absence of the ligand, second domain, and the R60P mutation. We further show that dynamics alone, independent of structural information, can be used to recover a network of interactions consistent with the contact map. Differences in dynamics and reconstructed interactions were used to identify regions on the truncated mutant that may explain functional differences in comparison to wild-type and full-length FimH.
Characterization of the metabotropic glutamate receptor conformational landscape

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The ability to sense external stimuli and dynamically respond lies at the core of cellular homeostasis. G protein-coupled receptors (GPCRs) are the largest family of membrane proteins in humans and function as key players in cellular signal processing. GPCRs are intrinsically allosteric proteins where ligand binding at the extracellular interface induces conformational changes that are then relayed to the intracellular G-protein-coupling region of the receptor. Therefore, understanding the activation mechanism of GPCRs requires quantifying the conformational dynamics of the receptors in a global context. Among all GPCRs, metabotropic glutamate receptors (mGluRs) are unique in that they form constitutive dimers and agonist binding is relayed over 120 Å to activate the receptors.

To understand how ligand-induced structural changes are propagated allosterically throughout the protein, we used a combination of single molecule Förster resonance energy transfer (smFRET) and live-cell imaging to visualize the propagation of conformational changes from the extracellular ligand binding domain (LBD) to the intracellular G protein coupling pocket. Our results show that the cysteine-rich domain (CRD) and heptahelical domain (HD) of mGluRs, both downstream of the LBD, can occupy several distinct conformations and transitions between these states occur in a sequential manner. Furthermore, we found the ligand-induced conformational dynamics among these three domains to be highly variable. These findings not only support recent work that describes a compaction of the intersubunit dimer interface upon receptor activation, but also more broadly supports the concept of loose coupling prevalent in the GPCR field by illustrating highly variable dynamic behavior among the LBD, CRD, and HD.
Precise Characterization and Comparison of KRAS Oncoproteoforms across Three Cancer Contexts

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RAS genes (KRAS, HRAS, NRAS) are among the most frequently mutated oncogenes in human cancer. The connections between RAS mutations, post-translational modifications (PTMs), and biological functions remain poorly understood, but can provide insight into the molecular mechanisms driving tumorigenesis. Due to high sequence identity (~90%), identification of each RAS isoform, or their respective mutations and PTMs, remains highly challenging for bottom-up proteomic approaches. Conversely, top-down proteomic (TDP) approaches are uniquely suited to precisely characterize intact modified forms, or proteoforms, of each RAS isoform.

We have developed a novel workflow to precisely characterize, quantify, and directly compare KRAS4B and KRAS4A proteoforms isolated from cancer cell lines and tumor samples by immunoprecipitation (IP) and subsequent analysis by top-down (TD) mass spectrometry on a QE-HF mass spectrometer (Figure 1). Previously, our IP-TD assay enabled the characterization of eleven KRAS4B proteoforms with complete molecular specificity from colorectal cancer cell lines and primary tumor samples. We have subsequently applied our IP-TD assay to the analysis of lower-abundance KRAS4B proteoforms from colorectal cancer cell lines on a 21 Tesla FT-ICR mass spectrometer. By significantly lowering the limit of detection and increasing the signal-to-noise ratio of each proteoform species, we have identified up to ten new KRAS4B proteoforms per context analyzed. Moreover, we have modified our IP-TD assay to incorporate KRAS4A, as we hypothesized that KRAS4B and KRAS4A would exhibit distinct proteoform populations both within and between cellular contexts. We have performed the first identification, characterization, and direct comparison of endogenous KRAS4B and KRAS4A proteoforms from two colorectal cancer cell lines on a QE-HF mass spectrometer, which led to the identification of four KRAS4A proteoforms and indicated possible differences in relative proteoform abundance between KRAS4A and KRAS4B. These latest advances have broadened our understanding of KRAS biology and provided new insights into the potential roles of KRAS proteoforms in colorectal, lung, and melanoma cancers.
Designing de novo αββα miniproteins using computational methods

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Computational protein design has been demonstrated to be an effective method in studying determinants of protein folding and stability. However, designs may not fold as predicted because the metrics used for the predictions may not exactly reflect the characteristics of natural proteins. Hence, repeated cycles of computational design improvement and experimental protein stability assays in a high-throughput manner can be a useful strategy to design stable proteins. Recently, Rocklin et al. (2017) designed and tested the stability of thousands of miniproteins whose secondary structures consisted of one of four topologies (HHH, EHEE, HEEH, and EEHEE). While HHH, EHEE, and EEHEE had success rates for stability of 87%, 39%, and 58%, respectively, HEEH had a surprisingly low success rate of 2%. We therefore interrogated why HEEH was unsuccessful and applied this new knowledge to generate new HEEH designs. We have currently identified computationally which design features correlated best with protein stability (e.g. overall hydrophobicity, buried non-polar residues), and are designing several thousand new HEEH backbones using Rosetta. Once we analyze the new designs, we will select a subset of designs and experimentally test them using yeast display proteolysis.
Structure of the membrane-bound, copper-dependent methane monooxygenase in a lipid environment

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Methanotrophs are methane-consuming bacteria that provide a promising solution to increasing worldwide emissions of methane, a potent greenhouse gas. Methanotrophs primarily use the copper-dependent membrane-bound enzyme particulate methane monooxygenase (pMMO) to convert methane to methanol as the first step in metabolizing methane. pMMO features two conserved metal binding sites whose roles in this reaction are under investigation. So far, efforts to express recombinant pMMO mutants have been unsuccessful. Cell-free protein expression offers the ability to study recombinantly expressed pMMO. In this approach, protein translation is decoupled from cell viability, allowing for the expression of otherwise toxic proteins in vitro. Using this system, pMMO is expressed directly into a membrane environment and mutants of amino acid residues at the conserved metal binding sites can be generated. Mutants with one metal binding site abolished can then be investigated using electron paramagnetic resonance, native mass spectrometry and activity by gas chromatography to determine the specific catalytic role of each site.

Another important aspect of this project involves the characterization of pMMO in a native-like lipid environment. To accomplish this, pMMO is reconstituted into a lipid nanodisc. The results of this characterization are used as benchmarks for pMMO produced by cell-free protein expression. Additionally, this formation of pMMO is ideal for structural investigation by cryo-electron microscopy. This technique is used to determine the structure of an active pMMO complex in a native-like lipid environment. This study will reveal the regulatory role of lipids in the activity of pMMO and may show more clearly electron densities that are not visible in the crystal structure.
Structural visualization of key intermediates in the NHEJ pathway by single particle cryo-EM

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Non-homologous end-joining (NHEJ) pathway is one of the major DNA repair pathways that repair DNA double strand breaks (DSBs) and maintain genome integrity throughout cell cycles. It repairs broken dsDNA by directly ligating the two DNA ends without any template. Although key repair factors involved in NHEJ have been identified and widely studied, less is known about the molecular mechanism of how these proteins cooperate in NHEJ to repair DSBs. Here, we applied single-particle cryo-electron microscopy (EM) to directly visualize key intermediate states of NHEJ repair machinery. By characterizing the architectures of NHEJ initiation complex (DNA-PK holoenzyme), Ligation complex (XRCC4, XLF and LigIV) and a novel super complex that consists both DNA-PK holoenzyme and Ligation complex, a working model of NHEJ mechanism is illustrated. Our structures indicate that DNA-PK holoenzyme, upon binding to DSB sites, dimerizes to bring two DNA ends to proximity, and recruit Ligase complex factors to the DNA repair loci, while the Ligase complex is responsible for both the alignment of fully synaptic dsDNA and the final ligation of two dsDNA ends. Our study is the first to comprehensively define the structural and functional role of important repair factors within the NHEJ machinery.